

WHAT IS CLAIMED IS:

1 1. An isolated infectious recombinant respiratory syncytial virus
2 (RSV) comprising a major nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P),
3 a large polymerase protein (L), a RNA polymerase elongation factor, and a partial or
4 complete recombinant RSV genome or antigenome having one or more shifted RSV
5 gene(s) or genome segment(s) within said recombinant genome or antigenome that is/are
6 positionally shifted to a more promoter-proximal or promoter-distal position relative to a
7 position of said RSV gene(s) or genome segment(s) within a wild type RSV genome or
8 antigenome.

1 2. The isolated infectious recombinant RSV of claim 1, wherein said
2 one or more shifted gene(s) or genome segment(s) is/are shifted to a more promoter-
3 proximal or promoter-distal position by deletion or insertion of one or more displacement
4 polynucleotide(s) within said partial or complete recombinant RSV genome or
5 antigenome.

1 3. The isolated infectious recombinant RSV of claim 2, wherein said
2 displacement polynucleotide(s) comprise(s) one or more polynucleotide insert(s) of
3 between 150 nucleotides (nts) and 4,000 nucleotides in length which is inserted in a non-
4 coding region (NCR) of the genome or antigenome or as a separate gene unit (GU), said
5 polynucleotide insert lacking a complete open reading frame (ORF) and specifying an
6 attenuated phenotype in said recombinant RSV.

1 4. The isolated infectious recombinant RSV of claim 3, wherein said
2 polynucleotide insert(s) comprises one or more RSV gene(s) or genome segment(s).

1 5. The isolated infectious recombinant RSV of claim 2, wherein said
2 displacement polynucleotide(s) comprise(s) one or more RSV gene(s) or genome
3 segment(s) selected from RSV NS1, NS2, N, P, M, SH, M2(ORF1), M2(ORF2), L, F and
4 G genes and genome segments and leader, trailer and intergenic regions of the RSV
5 genome and segments thereof.

1 6. The isolated infectious recombinant RSV of claim 2, wherein said
2 displacement polynucleotide(s) comprise(s) one or more bovine RSV (BRV) or human
3 RSV (HRSV) gene(s) or genome segment(s) selected from RSV NS1, NS2, N, P, M, SH,
4 M2(ORF1), M2(ORF2), L, F and G gene(s) or genome segment(s) and leader, trailer and
5 intergenic regions of the RSV genome or segments thereof.

1 7. The isolated infectious recombinant RSV of claim 6, wherein said
2 displacement polynucleotide(s) is/are deleted to form the recombinant RSV genome or
3 antigenome to cause a positional shift of said one or more shifted RSV gene(s) or genome
4 segment(s) within said recombinant genome or antigenome to a more promoter-proximal
5 position relative to a position of said RSV gene(s) or genome segment(s) within a wild
6 type RSV genome or antigenome.

1 8. The isolated infectious recombinant RSV of claim 7, wherein said
2 displacement polynucleotide(s) that is/are deleted to form the recombinant RSV genome
3 or antigenome comprise one or more RSV NS1, NS2, SH, M2(ORF2), or G gene(s) or
4 genome segment(s) thereof.

1 9. The isolated infectious recombinant RSV of claim 8, wherein a
2 displacement polynucleotide comprising a RSV NS1 gene is deleted to form the
3 recombinant RSV genome or antigenome.

1 10. The isolated infectious recombinant RSV of claim 8, wherein a
2 displacement polynucleotide comprising a RSV NS2 gene is deleted to form the
3 recombinant RSV genome or antigenome.

1 11. The isolated infectious recombinant RSV of claim 8, wherein a
2 displacement polynucleotide comprising a RSV SH gene is deleted to form the
3 recombinant RSV genome or antigenome.

1 12. The isolated infectious recombinant RSV of claim 8, wherein a
2 displacement polynucleotide comprising RSV M2(ORF2) is deleted to form the
3 recombinant RSV genome or antigenome.

1 13. The isolated infectious recombinant RSV of claim 8, wherein a
2 displacement polynucleotide comprising a RSV G gene is deleted to form the
3 recombinant RSV genome or antigenome or antigenome.

1 14. The isolated infectious recombinant RSV of claim 8, wherein the
2 RSV F and G genes are both deleted to form the recombinant RSV genome or
3 antigenome or antigenome.

1 15. The isolated infectious recombinant RSV of claim 8, wherein the
2 RSV NS1 and NS2 genes are both deleted to form the recombinant RSV genome or
3 antigenome or antigenome.

1 16. The isolated infectious recombinant RSV of claim 8, wherein the
2 RSV SH and NS2 genes are both deleted to form the recombinant RSV genome or
3 antigenome or antigenome.

1 17. The isolated infectious recombinant RSV of claim 8, wherein the
2 RSV SH, NS1 and NS2 genes are all deleted to form the recombinant RSV genome or
3 antigenome or antigenome.

1 18. The isolated infectious recombinant RSV of claim 7, wherein said
2 displacement polynucleotide(s) comprise(s) one or more deletion(s) within a
3 nontranslated sequence at the beginning or end of an RSV open reading frame or in an
4 intergenic region or 3' leader or 5' trailer portion of the RSV genome.

1 19. The isolated infectious recombinant RSV of claim 18, wherein said
2 displacement polynucleotides comprise a partial gene deletion.

1 20. The isolated infectious recombinant RSV of claim 19, wherein said
2 partial gene deletion is a partial deletion of the SH gene.

1 21. The isolated infectious recombinant RSV of claim 20, wherein said
2 partial deletion of the SH gene comprises a deletion within the SH downstream non-
3 translated region.

1 22. The isolated infectious recombinant RSV of claim 21, which is
2 RSV 6120 having a deletion of 112 nucleotides at positions 4499-4610 in the
3 recombinant RSV antigenome.

1 23. The isolated infectious recombinant RSV of claim 7, wherein said
2 displacement polynucleotide(s) is/are selected from one or more region(s) of a
3 downstream untranslated sequence of an RSV gene.

1 24. The isolated infectious recombinant RSV of claim 23, wherein said
2 downstream untranslated sequence(s) is/are from NS1 (positions 519-563), NS2
3 (positions 1003-1086), P (positions 3073-3230), M (positions 4033-4197), F(positions
4 7387-7539), and/or M2 (positions 8433-8490) genes.

1 25. The isolated infectious recombinant RSV of claim 7, wherein said
2 displacement polynucleotide(s) is/are selected from one or more region(s) of a upstream
3 untranslated sequence of an RSV gene.

1 26. The isolated infectious recombinant RSV of claim 25, wherein said
2 one or more upstream untranslated sequences is/are from NS1 (positions 55-96), NS2
3 (positions 606-624) and/or SH (positions 4231-4300).

1 27. The isolated infectious recombinant RSV of claim 7, wherein said
2 displacement polynucleotide comprises a deletion of nucleotides 4683 to 4685 of the RSV
3 G gene.

1 28. The isolated infectious recombinant RSV of claim 7, wherein said
2 displacement polynucleotide(s) is/are selected from one or more RSV intergenic
3 sequences.

1 29. The isolated infectious recombinant RSV of claim 7, wherein said
2 displacement polynucleotide(s) is/are selected from nucleotides within the RSV 5' trailer
3 region.

1 30. The isolated infectious recombinant RSV of claim 29, wherein a
2 portion of the 5' trailer region that immediately follows the L gene is reduced in size by
3 75 nucleotides, 100 nucleotides, 125 nucleotides, or more, leaving intact the 5' genomic
4 terminus.

1 31. The isolated infectious recombinant RSV of claim 7, wherein said
2 displacement polynucleotide(s) is/are selected from nucleotides within the RSV 3' leader
3 region.

1 32. The isolated infectious recombinant RSV of claim 31, wherein a
2 portion of the 3' trailer region that excludes a core portion of the viral promoter located
3 within the first 11 nucleotides of the 3' leader is deleted.

1 33. The isolated infectious recombinant RSV of claim 7, wherein a
2 partial or complete deletion from one or any combination of the RSV NS1, NS2, SH, F
3 and/or M2 genes yields an adjustable reduction in genome length of between 1-806
4 nucleotides.

1 34. The isolated infectious recombinant RSV of claim 7, wherein a
2 partial or complete deletion from one or any combination of RSV intergenic regions
3 yields an adjustable reduction in genome length of between 1-198 nucleotides.

1 35. The isolated infectious recombinant RSV of claim 7, wherein a
2 partial or complete deletion from one or any combination of RSV intergenic regions
3 yields an adjustable reduction in genome length of between 1-198 nucleotides.

1 36. The isolated infectious recombinant RSV of claim 6, wherein said
2 displacement polynucleotide(s) is/are added, substituted, or rearranged within the
3 recombinant RSV genome or antigenome to cause a positional shift of said one or more
4 shifted RSV gene(s) or genome segment(s) within said recombinant genome or
5 antigenome to a more promoter-proximal or promoter-distal position relative to a position
6 of said RSV gene(s) or genome segment(s) within a wild type RSV genome or
7 antigenome.

1 37. The isolated infectious recombinant RSV of claim 36, wherein said
2 displacement polynucleotide(s) added, substituted, or rearranged within the recombinant
3 RSV genome or antigenome comprise(s) one or more RSV NS1, NS2, SH, M2(ORF2), F,
4 and/or G gene(s) or genome segment(s) thereof.

1 38. The isolated infectious recombinant RSV of claim 36, wherein said
2 displacement polynucleotide(s) comprise(s) one or more RSV gene(s) or genome
3 segment(s) encoding one or more RSV glycoprotein(s) or immunogenic domain(s) or
4 epitope(s) thereof.

1 39. The isolated infectious recombinant RSV of claim 38, wherein said
2 displacement polynucleotide(s) is/are selected from gene(s) or genome segment(s)
3 encoding RSV F, G, and/or SH glycoprotein(s) or immunogenic domain(s) or epitope(s)
4 thereof.

1 40. The isolated infectious recombinant RSV of claim 1, wherein one
2 or more RSV glycoprotein gene(s) or genome segments of RSV F, G and SH is/are
3 added, substituted or rearranged within said recombinant RSV genome or antigenome to a
4 position that is more promoter-proximal compared to a wild type gene order position of
5 said one or more RSV glycoprotein gene(s).

1 41. The isolated infectious recombinant RSV of claim 40, wherein the
2 RSV glycoprotein gene G is rearranged within said recombinant RSV genome or
3 antigenome to a gene order position that is more promoter-proximal compared to the wild
4 type gene order position of G.

1 42. The isolated infectious recombinant RSV of claim 41, wherein the
2 RSV glycoprotein gene G is shifted to gene order position 1 within said recombinant RSV
3 genome or antigenome.

1 43. The isolated infectious recombinant RSV of claim 40, wherein the
2 RSV glycoprotein gene F is rearranged within said recombinant RSV genome or

3 antigenome to a gene order position that is more promoter-proximal compared to the wild
4 type gene order position of F.

1 44. The isolated infectious recombinant RSV of claim 43, wherein the
2 RSV glycoprotein gene F is shifted to gene order position 1 within said recombinant RSV
3 genome or antigenome.

1 45. The isolated infectious recombinant RSV of claim 40, wherein both
2 RSV glycoprotein genes G and F are rearranged within said recombinant RSV genome or
3 antigenome to gene order positions that are more promoter-proximal compared to the
4 wild type gene order positions of G and F.

1 46. The isolated infectious recombinant RSV of claim 45, wherein the
2 RSV glycoprotein gene G is shifted to gene order position 1 and the RSV glycoprotein
3 gene F is shifted to gene order position 2 within said recombinant RSV genome or
4 antigenome.

1 47. The isolated infectious recombinant RSV of claim 40, wherein one
2 or more RSV NS1, NS2, SH, M2(ORF2), or G gene(s) or genome segment(s) thereof
3 is/are deleted in the recombinant RSV genome or antigenome.

1 48. The isolated infectious recombinant RSV of claim 40, wherein a
2 displacement polynucleotide comprising a RSV NS1 gene is deleted to form the
3 recombinant RSV genome or antigenome.

1 49. The isolated infectious recombinant RSV of claim 40, wherein a
2 displacement polynucleotide comprising a RSV NS2 gene is deleted to form the
3 recombinant RSV genome or antigenome.

1 50. The isolated infectious recombinant RSV of claim 40 wherein a
2 displacement polynucleotide comprising a RSV SH gene is deleted to form the
3 recombinant RSV genome or antigenome.

1 51. The isolated infectious recombinant RSV of claim 50, wherein the
2 RSV glycoprotein gene G is rearranged within said recombinant RSV genome or

3 antigenome to a gene order position that is more promoter-proximal compared to the wild
4 type gene order position of G.

1 52. The isolated infectious recombinant RSV of claim 51, wherein the
2 RSV glycoprotein gene G is shifted to gene order position 1 within said recombinant RSV
3 genome or antigenome.

1 53. The isolated infectious recombinant RSV of claim 52, which is
2 G1/ Δ SH.

1 54. The isolated infectious recombinant RSV of claim 50, wherein the
2 RSV glycoprotein gene F is rearranged within said recombinant RSV genome or
3 antigenome to a gene order position that is more promoter-proximal compared to the wild
4 type gene order position of F.

1 55. The isolated infectious recombinant RSV of claim 54, wherein the
2 RSV glycoprotein gene F is shifted to gene order position 1 within said recombinant RSV
3 genome or antigenome.

1 56. The isolated infectious recombinant RSV of claim 55, which is
2 F1/ Δ SH.

1 57. The isolated infectious recombinant RSV of claim 50, wherein both
2 RSV glycoprotein genes G and F are rearranged within said recombinant RSV genome or
3 antigenome to gene order positions that are more promoter-proximal compared to the
4 wild type gene order positions of G and F.

1 58. The isolated infectious recombinant RSV of claim 57, wherein the
2 RSV glycoprotein gene G is shifted to gene order position 1 and the RSV glycoprotein
3 gene F is shifted to gene order position 2 within said recombinant RSV genome or
4 antigenome.

1 59. The isolated infectious recombinant RSV of claim 58, which is
2 G1F2/ Δ SH.

1 60. The isolated infectious recombinant RSV of claim 40, wherein the
2 RSV SH and NS2 genes are both deleted to form the recombinant RSV genome or
3 antigenome or antigenome.

1 61. The isolated infectious recombinant RSV of claim 60, wherein both
2 RSV glycoprotein genes G and F are rearranged within said recombinant RSV genome or
3 antigenome to gene order positions that are more promoter-proximal compared to the
4 wild type gene order positions of G and F.

1 62. The isolated infectious recombinant RSV of claim 61, wherein the
2 RSV glycoprotein gene G is shifted to gene order position 1 and the RSV glycoprotein
3 gene F is shifted to gene order position 2 within said recombinant RSV genome or
4 antigenome.

1 63. The isolated infectious recombinant RSV of claim 62, which is
2 G1F2/ Δ NS2 Δ SH.

1 64. The isolated infectious recombinant RSV of claim 40, wherein the
2 RSV SH, NS1 and NS2 genes are all deleted to form the recombinant RSV genome or
3 antigenome or antigenome.

1 65. The isolated infectious recombinant RSV of claim 64, wherein both
2 RSV glycoprotein genes G and F are rearranged within said recombinant RSV genome or
3 antigenome to gene order positions that are more promoter-proximal compared to the
4 wild type gene order positions of G and F.

1 66. The isolated infectious recombinant RSV of claim 65, wherein the
2 RSV glycoprotein gene G is shifted to gene order position 1 and the RSV glycoprotein
3 gene F is shifted to gene order position 2 within said recombinant RSV genome or
4 antigenome.

1 67. The isolated infectious recombinant RSV of claim 66, which is
2 G1F2/ Δ NS2 Δ NS2 Δ SH.

1 68. The isolated infectious recombinant RSV of claim 1, wherein the
2 recombinant genome or antigenome comprises a partial or complete human RSV (HRSV)
3 or bovine RSV (BRSV) background genome or antigenome combined with one or more
4 heterologous gene(s) or genome segment(s) from a different RSV to form a human-
5 bovine chimeric RSV genome or antigenome.

1 69. The isolated infectious recombinant RSV of claim 68, wherein the
2 heterologous gene or genome segment is added or substituted at a position that is more
3 promoter-proximal or promoter-distal compared to a wild type gene order position of a
4 counterpart gene or genome segment within the partial or complete HRSV or BRSV
5 background genome or antigenome.

1 70. The isolated infectious recombinant RSV of claim 69, wherein both
2 human RSV glycoprotein genes G and F are substituted at gene order positions 1 and 2,
3 respectively, to replace counterpart G and F glycoprotein genes deleted at wild type
4 positions 7 and 8, respectively in a partial bovine RSV background genome or
5 antigenome.

1 71. The isolated infectious recombinant RSV of claim 70, which is
2 rBRSV/A2-G1F2.

1 72. The isolated infectious recombinant RSV of claim 69, wherein one
2 or more human RSV non-structural and/or envelope-associated genes selected from NS1,
3 NS2, F, G, SH, and M is/are added or substituted within a partial or complete bovine RSV
4 background genome or antigenome.

1 73. The isolated infectious recombinant RSV of claim 69, wherein one
2 or more human RSV envelope-associated genes selected from F, G, SH, and M is/are
3 added or substituted within a partial bovine RSV background genome or antigenome in
4 which one or more envelope-associated genes selected from F, G, SH, and M is/are
5 deleted.

1 74. The isolated infections recombinant RSV of claim 73, wherein
2 human RSV envelope-associated genes F, G, and M are added within a partial bovine
3 RSV background genome or antigenome in which all of the envelope-associated genes F,
4 G, SH, and M are deleted.

1 75. The isolated infectious recombinant RSV of claim 74, which is
2 rBRSV/A2-MGF.

1 76. The isolated infectious recombinant RSV of claim 69, wherein both
2 human RSV glycoprotein genes G and F are substituted at gene order positions 3 and 4,
3 respectively, to replace counterpart G and F glycoprotein genes deleted at wild type
4 positions 7 and 8, respectively in a partial bovine RSV background genome or
5 antigenome.

1 77. The isolated infectious recombinant RSV of claim 76, which is
2 rBRSV/A2-G3F4.

1 78. The isolated infectious recombinant RSV of claim 69, wherein both
2 human RSV glycoprotein genes G and F are substituted at gene order positions 1 and 2,
3 respectively, to replace counterpart G and F glycoprotein genes deleted at wild type
4 positions 7 and 8, respectively, and wherein human RSV genes NS1 and NS2 are
5 substituted for their bovine counterpart genes, in a partial bovine RSV background
6 genome or antigenome.

1 79. The isolated infectious recombinant RSV of claim 78, which is
2 rBRSV/A2-G1F2NS3NS4.

1 80. The isolated infectious recombinant RSV of claim 1, in which RSV
2 M2(ORF1) is shifted to a more promoter-proximal position within the recombinant RSV
3 genome or antigenome to upregulate transcription of the recombinant virus.

1 81. The isolated infectious recombinant RSV of claim 1, wherein the
2 recombinant genome or antigenome incorporates at least one and up to a full complement
3 of attenuating mutations present within a panel of mutant human RSV strains, said panel

4 comprising cpts RSV 248 (ATCC VR 2450), cpts RSV 248/404 (ATCC VR 2454), cpts
5 RSV 248/955 (ATCC VR 2453), cpts RSV 530 (ATCC VR 2452), cpts RSV 530/1009
6 (ATCC VR 2451), cpts RSV 530/1030 (ATCC VR 2455), RSV B-1 cp52/2B5 (ATCC
7 VR 2542), and RSV B-1 cp-23 (ATCC VR 2579).

1 82. The isolated infectious recombinant RSV of claim 81, wherein the
2 recombinant genome or antigenome incorporates attenuating mutations adopted from
3 different mutant RSV strains.

1 83. The isolated infectious recombinant RSV of claim 1, wherein the
2 recombinant genome or antigenome incorporates at least one and up to a full complement
3 of attenuating mutations specifying an amino acid substitution at Val267 in the RSV N
4 gene, Glu218 and/or Thr523 in the RSV F gene, Asn43, Cys319, Phe 521, Gln831,
5 Met1169, Tyr1321 and/or His 1690 in the RSV polymerase gene L, and a nucleotide
6 substitution in the gene-start sequence of gene M2.

1 84. The isolated infectious recombinant RSV of claim 83, wherein the
2 recombinant genome or antigenome incorporates at least two attenuating mutations.

1 85. The isolated infectious recombinant RSV of claim 83, wherein the
2 recombinant genome or antigenome includes at least one attenuating mutation stabilized
3 by multiple nucleotide changes in a codon specifying the mutation.

1 86. The isolated infectious recombinant RSV of claim 1, wherein the
2 recombinant genome or antigenome further comprises a nucleotide modification
3 specifying a phenotypic change selected from a change in growth characteristics,
4 attenuation, temperature-sensitivity, cold-adaptation, plaque size, host-range restriction,
5 or a change in immunogenicity.

1 87. The isolated infectious recombinant RSV of claim 86, wherein the
2 nucleotide modification alters a SH, NS1, NS2, M2ORF2, or G gene of the recombinant
3 virus.

1 88. The isolated infectious recombinant RSV of claim 87, wherein a
2 SH, NS1, NS2, M2 ORF2, or G gene of the recombinant virus is deleted in whole or in
3 part or expression of the gene is ablated by introduction of one or more stop codons in an
4 open reading frame of the gene.

1 89. The isolated infectious recombinant RSV of claim 86, wherein the
2 nucleotide modification comprises a nucleotide deletion, insertion, substitution, addition
3 or rearrangement of a cis-acting regulatory sequence of a selected gene within the
4 recombinant RSV genome or antigenome.

1 90. The isolated infectious recombinant RSV of claim 89, wherein a
2 gene end (GE) signal of the NS1 or NS2 gene is modified.

1 91. The isolated infectious recombinant RSV of claim 89, wherein the
2 nucleotide modification comprises an insertion, deletion, substitution, or rearrangement of
3 a translational start site within the recombinant genome or antigenome.

1 92. The isolated infectious recombinant RSV of claim 91, wherein the
2 translational start site for a secreted form of the RSV G glycoprotein is ablated.

1 93. The isolated infectious recombinant RSV of claim 1, wherein the
2 recombinant genome or antigenome is modified to encode a non-RSV molecule selected
3 from a cytokine, a T-helper epitope, a restriction site marker, or a protein of a microbial
4 pathogen capable of eliciting a protective immune response against said pathogen in a
5 mammalian host.

1 94. The isolated infectious recombinant RSV of claim 93, which
2 incorporates one or more gene(s) and/or genome segment(s) from parainfluenza virus
3 (PIV).

1 95. The isolated infectious recombinant RSV of claim 94, wherein the
2 recombinant genome or antigenome encodes a HN or F glycoprotein, or an ectodomain or
3 immunogenic epitope of HN or F, of PIV1, PIV2, or PIV3.

- 1 96. The isolated infectious recombinant RSV of claim 1 which is a
2 virus.
- 1 97. The isolated infectious recombinant RSV of claim 1 which is a
2 subviral particle.
- 1 98. The isolated infectious recombinant RSV of claim 2, wherein said
2 displacement polynucleotide is added within or deleted from a noncoding region of the
3 recombinant RSV genome or antigenome.
- 1 99. The isolated infectious recombinant RSV of claim 1, wherein the
2 recombinant genome or antigenome incorporates antigenic determinants from one or both
3 subgroup A and subgroup B human RSV.
- 1 100. A method for stimulating the immune system of an individual to
2 induce protection against RSV which comprises administering to the individual an
3 immunologically sufficient amount of the recombinant RSV of claim 1 combined with a
4 physiologically acceptable carrier.
- 1 101. The method of claim 100, wherein the recombinant RSV is
2 administered in a dose of 10^3 to 10^6 PFU.
- 1 102. The method of claim 100, wherein the recombinant RSV is
2 administered to the upper respiratory tract.
- 1 103. The method of claim 100, wherein the recombinant RSV is
2 administered by spray, droplet or aerosol.
- 1 104. The method of claim 100, wherein the recombinant RSV is
2 administered to an individual seronegative for antibodies to RSV or possessing
3 transplacentally acquired maternal antibodies to RSV.
- 1 105. The method of claim 100, wherein the recombinant RSV elicits an
2 immune response against either human RSV A or RSV B.

09887469-062301
102290 6942850

1 106. The method of claim 100, wherein the recombinant RSV elicits an
2 immune response against both human RSV A and RSV B.

1 107. The method of claim 100, wherein the recombinant RSV elicits an
2 immune response against either human RSV A or RSV B and is co-administered with an
3 immunologically sufficient amount of a second attenuated RSV capable of eliciting an
4 immune response against human RSV A or RSV B, whereby an immune response is
5 elicited against both human RSV A and RSV B.

1 108. The method of claim 107, wherein the recombinant RSV and
2 second attenuated RSV are administered simultaneously as a mixture.

1 109. An immunogenic composition to elicit an immune response against
2 RSV comprising an immunologically sufficient amount of the recombinant RSV of claim
3 1 in a physiologically acceptable carrier.

1 110. The immunogenic composition of claim 109, formulated in a dose
2 of 10^3 to 10^6 PFU.

1 111. The immunogenic composition of claim 109, formulated for
2 administration to the upper respiratory tract by spray, droplet or aerosol.

1 112. The immunogenic composition of claim 109, wherein the
2 recombinant RSV elicits an immune response against either human RSV A or RSV B or
3 both human RSV A and RSV B.

1 113. The isolated infectious recombinant RSV of claim 1, wherein the
2 recombinant genome or antigenome comprises a partial or complete RSV vector genome
3 or antigenome combined with one or more heterologous genes or genome segments
4 encoding one or more antigenic determinants of one or more heterologous pathogens.

1 114. The isolated infectious recombinant RSV of claim 113, wherein
2 said one or more heterologous pathogens is a heterologous RSV and said heterologous

3 gene(s) or genome segment(s) encode(s) one or more RSV NS1, NS2, N, P, M, SH,
4 M2(ORF1), M2(ORF2), L, F or G protein(s) or fragment(s) thereof.

1 115. The isolated infectious recombinant RSV of claim 113, wherein the
2 vector genome or antigenome is a partial or complete RSV A genome or antigenome and
3 the heterologous gene(s) or genome segment(s) encoding the antigenic determinant(s)
4 is/are of a RSV B subgroup virus.

1 116. The isolated infectious recombinant RSV of claim 113, wherein the
2 chimeric genome or antigenome incorporates one or more gene(s) or genome segment(s)
3 of a BRSV that specifies attenuation.

1 117. The isolated infectious recombinant RSV of claim 113, wherein
2 one or more HPIV1, HPIV2, or HPIV3 gene(s) or genome segment(s) encoding one or
3 more HN and/or F glycoprotein(s) or antigenic domain(s), fragment(s) or epitope(s)
4 thereof is/are added to or incorporated within the partial or complete HRSV vector
5 genome or antigenome.

1 118. The isolated infectious recombinant RSV of claim 113, wherein the
2 vector genome or antigenome is a partial or complete BRSV genome or antigenome and
3 the heterologous gene(s) or genome segment(s) encoding the antigenic determinant(s)
4 is/are of one or more HRSV(s).

1 119. The isolated infectious recombinant RSV of claim 118, wherein the
2 partial or complete BRSV genome or antigenome incorporates one or more gene(s) or
3 genome segment(s) encoding one or more HRSV glycoprotein genes selected from F, G
4 and SH, or one or more genome segment(s) encoding cytoplasmic domain,
5 transmembrane domain, ectodomain or immunogenic epitope portion(s) of F, G, and/or
6 SH of HRSV.

1 120. The isolated infectious recombinant RSV of claim 113, wherein the
2 vector genome or antigenome is a partial or complete HRSV or BRSV genome or
3 antigenome and the heterologous pathogen is selected from measles virus, subgroup A
4 and subgroup B respiratory syncytial viruses, mumps virus, human papilloma viruses,
5 type 1 and type 2 human immunodeficiency viruses, herpes simplex viruses,

6 cytomegalovirus, rabies virus, Epstein Barr virus, filoviruses, bunyaviruses, flaviviruses,
7 alphaviruses and influenza viruses.

1 121. The isolated infectious recombinant RSV of claim 120, wherein
2 said one or more heterologous antigenic determinant(s) is/are selected from measles virus
3 HA and F proteins, subgroup A or subgroup B respiratory syncytial virus F, G, SH and
4 M2 proteins, mumps virus HN and F proteins, human papilloma virus L1 protein, type 1
5 or type 2 human immunodeficiency virus gp160 protein, herpes simplex virus and
6 cytomegalovirus gB, gC, gD, gE, gG, gH, gI, gJ, gK, gL, and gM proteins, rabies virus G
7 protein, Epstein Barr Virus gp350 protein; filovirus G protein, bunyavirus G protein,
8 Flavivirus E and NS1 proteins, and alphavirus E protein, and antigenic domains,
9 fragments and epitopes thereof.

1 122. The isolated infectious recombinant RSV of claim 121, wherein the
2 heterologous pathogen is measles virus and the heterologous antigenic determinant(s)
3 is/are selected from the measles virus HA and F proteins and antigenic domains,
4 fragments and epitopes thereof.

1 123. The isolated infectious recombinant RSV of claim 122, wherein a
2 transcription unit comprising an open reading frame (ORF) of a measles virus HA gene is
3 added to or incorporated within a HRSV vector genome or antigenome.

1 124. An isolated polynucleotide molecule comprising a recombinant
2 RSV genome or antigenome having one or more shifted RSV gene(s) or genome
3 segment(s) within said recombinant genome or antigenome that is/are positionally shifted
4 to a more promoter-proximal or promoter-distal position relative to a position of said
5 RSV gene(s) or genome segment(s) within a wild type RSV genome or antigenome.

1 125. The isolated polynucleotide molecule of claim 124, wherein said
2 one or more shifted gene(s) or genome segment(s) is/are shifted to a more promoter-
3 proximal position by insertion or deletion of one or more displacement polynucleotide(s)
4 within said partial or complete recombinant RSV genome or antigenome.

1 126. The isolated polynucleotide molecule of claim 125, wherein said
2 displacement polynucleotide(s) comprise(s) one or more polynucleotide insert(s) of
3 between 150 nucleotides (nts) and 4,000 nucleotides in length which is inserted in a non-

4 coding region (NCR) of the genome or antigenome or as a separate gene unit (GU), said
5 polynucleotide insert lacking a complete open reading frame (ORF) and specifying an
6 attenuated phenotype in said recombinant RSV.

1 127. The isolated polynucleotide molecule of claim 126, wherein said
2 polynucleotide insert(s) comprises one or more RSV gene(s) or genome segment(s).

1 128. The isolated polynucleotide molecule of claim 127, wherein said
2 displacement polynucleotide(s) comprise(s) one or more bovine RSV (BRSV) or human
3 RSV (HRSV) gene(s) or genome segment(s) selected from RSV NS1, NS2, N, P, M, SH,
4 M2(ORF1), M2(ORF2), L, F and G gene(s) or genome segment(s) and leader, trailer and
5 intergenic regions of the RSV genome or segments thereof.

1 129. The isolated polynucleotide molecule of claim 128, wherein said
2 displacement polynucleotide(s) is/are deleted to form the recombinant RSV genome or
3 antigenome to cause a positional shift of said one or more shifted RSV gene(s) or genome
4 segment(s) within said recombinant genome or antigenome to a more promoter-proximal
5 position relative to a position of said RSV gene(s) or genome segment(s) within a wild
6 type RSV genome or antigenome.

1 130. The isolated polynucleotide molecule of claim 129, wherein said
2 displacement polynucleotide(s) that is/are deleted to form the recombinant RSV genome
3 or antigenome comprise one or more RSV NS1, NS2, SH, M2(ORF2), or G gene(s) or
4 genome segment(s) thereof.

1 131. The isolated polynucleotide molecule of claim 130, wherein a
2 displacement polynucleotide comprising a RSV NS1 gene is deleted to form the
3 recombinant RSV genome or antigenome.

1 132. The isolated polynucleotide molecule of claim 130, wherein a
2 displacement polynucleotide comprising a RSV NS2 gene is deleted to form the
3 recombinant RSV genome or antigenome.

1 133. The isolated polynucleotide molecule of claim 130, wherein a
2 displacement polynucleotide comprising a RSV SH gene is deleted to form the
3 recombinant RSV genome or antigenome.

- 1 134. The isolated polynucleotide molecule of claim 130, wherein a
2 displacement polynucleotide comprising RSV M2(ORF2) is deleted to form the
3 recombinant RSV genome or antigenome.
- 1 135. The isolated polynucleotide molecule of claim 130, wherein a
2 displacement polynucleotide comprising a RSV G gene is deleted to form the
3 recombinant RSV genome or antigenome or antigenome.
- 1 136. The isolated polynucleotide molecule of claim 130, wherein the
2 RSV F and G genes are both deleted to form the recombinant RSV genome or
3 antigenome or antigenome.
- 1 137. The isolated polynucleotide molecule of claim 130, wherein the
2 RSV NS1 and NS2 genes are both deleted to form the recombinant RSV genome or
3 antigenome or antigenome.
- 1 138. The isolated polynucleotide molecule of claim 130, wherein the
2 RSV SH and NS2 genes are both deleted to form the recombinant RSV genome or
3 antigenome or antigenome.
- 1 139. The isolated polynucleotide molecule of claim 130, wherein the
2 RSV SH, NS1 and NS2 genes are all deleted to form the recombinant RSV genome or
3 antigenome or antigenome.
- 1 140. The isolated polynucleotide molecule of claim 128, wherein said
2 displacement polynucleotide(s) is/are added, substituted, or rearranged within the
3 recombinant RSV genome or antigenome to cause a positional shift of said one or more
4 shifted RSV gene(s) or genome segment(s) within said recombinant genome or
5 antigenome to a more promoter-proximal or promoter-distal position relative to a position
6 of said RSV gene(s) or genome segment(s) within a wild type RSV genome or
7 antigenome.
- 1 141. The isolated polynucleotide molecule of claim 140, wherein said
2 displacement polynucleotide(s) added, substituted, or rearranged within the recombinant
3 RSV genome or antigenome comprise(s) one or more RSV NS1, NS2, SH, M2(ORF2), F,
4 and/or G gene(s) or genome segment(s) thereof.

1 142. The isolated polynucleotide molecule of claim 140, wherein said
2 displacement polynucleotide(s) comprise(s) one or more RSV gene(s) or genome
3 segment(s) encoding one or more RSV glycoprotein(s) or immunogenic domain(s) or
4 epitope(s) thereof.

1 143. The isolated polynucleotide molecule of claim 141, wherein said
2 displacement polynucleotide(s) is/are selected from gene(s) or genome segment(s)
3 encoding RSV F, G, and/or SH glycoprotein(s) or immunogenic domain(s) or epitope(s)
4 thereof.

1 144. The isolated polynucleotide molecule of claim 143, wherein one or
2 more RSV glycoprotein gene(s) selected from F, G and SH is/are added, substituted or
3 rearranged within said recombinant RSV genome or antigenome to a position that is more
4 promoter-proximal compared to a wild type gene order position of said one or more RSV
5 glycoprotein gene(s).

1 145. The isolated polynucleotide molecule of claim 144, wherein the
2 RSV glycoprotein gene G is rearranged within said recombinant RSV genome or
3 antigenome to a gene order position that is more promoter-proximal compared to the wild
4 type gene order position of G.

1 146. The isolated polynucleotide molecule of claim 145, wherein the
2 RSV glycoprotein gene G is shifted to gene order position 1 within said recombinant RSV
3 genome or antigenome.

1 147. The isolated polynucleotide molecule of claim 144, wherein the
2 RSV glycoprotein gene F is rearranged within said recombinant RSV genome or
3 antigenome to a gene order position that is more promoter-proximal compared to the wild
4 type gene order position of F.

1 148. The isolated polynucleotide molecule of claim 147, wherein the
2 RSV glycoprotein gene F is shifted to gene order position 1 within said recombinant RSV
3 genome or antigenome.

1 149. The isolated polynucleotide molecule of claim 144, wherein both
2 RSV glycoprotein genes G and F are rearranged within said recombinant RSV genome or

US87469-062201

3 antigenome to gene order positions that are more promoter-proximal compared to the
4 wild type gene order positions of G and F.

1 150. The isolated polynucleotide molecule of claim 149, wherein the
2 RSV glycoprotein gene G is shifted to gene order position 1 and the RSV glycoprotein
3 gene F is shifted to gene order position 2 within said recombinant RSV genome or
4 antigenome.

1 151. The isolated polynucleotide molecule of claim 144, wherein one or
2 more RSV NS1, NS2, SH, M2(ORF2), or G gene(s) or genome segment(s) thereof is/are
3 deleted in the recombinant RSV genome or antigenome.

1 152. The isolated polynucleotide molecule of claim 144, wherein a
2 displacement polynucleotide comprising a RSV NS1 gene is deleted to form the
3 recombinant RSV genome or antigenome.

1 153. The isolated polynucleotide molecule of claim 144, wherein a
2 displacement polynucleotide comprising a RSV NS2 gene is deleted to form the
3 recombinant RSV genome or antigenome.

1 154. The isolated polynucleotide molecule of claim 144 wherein a
2 displacement polynucleotide comprising a RSV SH gene is deleted to form the
3 recombinant RSV genome or antigenome.

1 155. The isolated polynucleotide molecule of claim 154, wherein the
2 RSV glycoprotein gene G is rearranged within said recombinant RSV genome or
3 antigenome to a gene order position that is more promoter-proximal compared to the wild
4 type gene order position of G.

1 156. The isolated polynucleotide molecule of claim 155, wherein the
2 RSV glycoprotein gene G is shifted to gene order position 1 within said recombinant RSV
3 genome or antigenome.

1 157. The isolated polynucleotide molecule of claim 154, wherein the
2 RSV glycoprotein gene F is rearranged within said recombinant RSV genome or
3 antigenome to a gene order position that is more promoter-proximal compared to the wild
4 type gene order position of F.

1 158. The isolated polynucleotide molecule of claim 157, wherein the
2 RSV glycoprotein gene F is shifted to gene order position 1 within said recombinant RSV
3 genome or antigenome.

1 159. The isolated polynucleotide molecule of claim 158, which is
2 F1/ΔSH.

1 160. The isolated polynucleotide molecule of claim 154, wherein both
2 RSV glycoprotein genes G and F are rearranged within said recombinant RSV genome or
3 antigenome to gene order positions that are more promoter-proximal compared to the
4 wild type gene order positions of G and F.

1 161. The isolated polynucleotide molecule of claim 160, wherein the
2 RSV glycoprotein gene G is shifted to gene order position 1 and the RSV glycoprotein
3 gene F is shifted to gene order position 2 within said recombinant RSV genome or
4 antigenome.

1 162. The isolated polynucleotide molecule of claim 144, wherein the
2 RSV SH and NS2 genes are both deleted to form the recombinant RSV genome or
3 antigenome or antigenome.

1 163. The isolated polynucleotide molecule of claim 162, wherein both
2 RSV glycoprotein genes G and F are rearranged within said recombinant RSV genome or
3 antigenome to gene order positions that are more promoter-proximal compared to the
4 wild type gene order positions of G and F.

1 164. The isolated polynucleotide molecule of claim 163, wherein the
2 RSV glycoprotein gene G is shifted to gene order position 1 and the RSV glycoprotein
3 gene F is shifted to gene order position 2 within said recombinant RSV genome or
4 antigenome.

1 165. The isolated polynucleotide molecule of claim 164, wherein the
2 RSV SH, NS1 and NS2 genes are all deleted to form the recombinant RSV genome or
3 antigenome or antigenome.

1 166. The isolated polynucleotide molecule of claim 165, wherein both
2 RSV glycoprotein genes G and F are rearranged within said recombinant RSV genome or
3 antigenome to gene order positions that are more promoter-proximal compared to the
4 wild type gene order positions of G and F.

1 167. The isolated polynucleotide molecule of claim 166, wherein the
2 RSV glycoprotein gene G is shifted to gene order position 1 and the RSV glycoprotein
3 gene F is shifted to gene order position 2 within said recombinant RSV genome or
4 antigenome.

1 168. The isolated polynucleotide of claim 124, wherein the recombinant
2 genome or antigenome comprises a partial or complete human or bovine RSV
3 background genome or antigenome combined with one or more heterologous gene(s)
4 and/or genome segment(s) from a different RSV to form a human-bovine chimeric
5 genome or antigenome.

1 169. The isolated polynucleotide of claim 168, wherein one or both
2 human RSV glycoprotein genes F and G is/are substituted to replace one or both
3 counterpart F and G glycoprotein genes in a partial bovine RSV background genome or
4 antigenome.

1 170. The isolated polynucleotide of claim 169, wherein both human
2 RSV glycoprotein genes F and G are substituted to replace counterpart F and G
3 glycoprotein genes in the bovine RSV background genome or antigenome.

1 171. The isolated polynucleotide of claim 168, wherein one or more
2 human RSV glycoprotein genes selected from F, G and SH is/are added or substituted at a
3 position that is more promoter-proximal compared to a wild-type gene order position of a
4 counterpart gene or genome segment within a partial or complete bovine RSV
5 background genome or antigenome.

1 172. The isolated polynucleotide of claim 171, wherein both human
2 RSV glycoprotein genes G and F are substituted at gene order positions 1 and 2,
3 respectively, to replace counterpart G and F glycoprotein genes deleted at wild type

4 positions 7 and 8, respectively in a partial bovine RSV background genome or
5 antigenome.

1 173. The isolated polynucleotide of claim 124, wherein the recombinant
2 genome or antigenome is further modified by addition or substitution of one or more
3 additional heterologous gene(s) or genome segment(s) from a human RSV within the
4 partial or complete bovine background genome or antigenome to increase genetic stability
5 or alter attenuation, reactogenicity or growth in culture of the recombinant virus.

1 174. The isolated polynucleotide molecule of claim 124, wherein the
2 recombinant genome or antigenome incorporates antigenic determinants from both
3 subgroup A and subgroup B human RSV.

1 175. The isolated polynucleotide molecule of claim 124, wherein the
2 recombinant genome or antigenome is further modified by incorporation of one or more
3 attenuating mutations.

1 176. The isolated polynucleotide molecule of claim 124, wherein the
2 recombinant genome or antigenome is further modified by incorporation of a nucleotide
3 modification specifying a phenotypic change selected from a change in growth
4 characteristics, attenuation, temperature-sensitivity, cold-adaptation, plaque size, host-
5 range restriction, or a change in immunogenicity.

1 177. The isolated polynucleotide molecule of claim 176, wherein a SH,
2 NS1, NS2, M2ORF2, or G gene is modified.

1 178. The isolated polynucleotide molecule of claim 177, wherein the
2 SH, NS1, NS2, M2 ORF2, or G gene is deleted in whole or in part or expression of the
3 gene is ablated by introduction of one or more stop codons in an open reading frame of
4 the gene.

1 179. The isolated polynucleotide molecule of claim 176, wherein the
2 nucleotide modification comprises a nucleotide deletion, insertion, addition or
3 rearrangement of a cis-acting regulatory sequence of a selected RSV gene within the
4 recombinant RSV genome or antigenome.

1 180. The isolated polynucleotide molecule of claim 124, wherein said
2 displacement polynucleotide(s) comprise(s) one or more deletion(s) within a
3 nontranslated sequence at the beginning or end of an RSV open reading frame or in an
4 intergenic region or 3' leader or 5' trailer portion of the RSV genome.

1 181. The isolated polynucleotide molecule of claim 180, wherein said
2 displacement polynucleotides comprise or partial gene deletion.

1 182. The isolated polynucleotide molecule of claim 181, wherein said
2 partial gene deletion is a partial deletion of the SH gene.

1 183. The isolated polynucleotide molecule of claim 182, wherein said
2 partial deletion of the SH gene comprises a deletion within the SH downstream non-
3 translated region.

1 184. The isolated polynucleotide molecule of claim 183, which is RSV
2 6120 having a deletion of 112 nucleotides at positions 4499-4610 in the recombinant
3 RSV antigenome.

1 185. The isolated polynucleotide molecule of claim 124, wherein said
2 displacement polynucleotide(s) is/are selected from one or more region(s) of a
3 downstream untranslated sequence of an RSV gene.

1 186. The isolated polynucleotide molecule of claim 185, wherein said
2 downstream untranslated sequence(s) is/are from NS1 (positions 519-563), NS2
3 (positions 1003-1086), P (positions 3073-3230), M (positions 4033-4197), F(positions
4 7387-7539), and/or M2 (positions 8433-8490) genes.

1 187. The isolated polynucleotide molecule of claim 124, wherein said
2 displacement polynucleotide(s) is/are selected from one or more region(s) of a upstream
3 untranslated sequence of an RSV gene.

1 188. The isolated polynucleotide molecule of claim 187, wherein said
2 one or more upstream untranslated sequences is/are from NS1 (positions 55-96), NS2
3 (positions 606-624) and/or SH (positions 4231-4300).

1 189. The isolated polynucleotide molecule of claim 124, wherein said
2 displacement polynucleotide comprises a deletion of nucleotides 4683 to 4685 of the RSV
3 G gene.

1 190. The isolated polynucleotide molecule of claim 124, wherein said
2 displacement polynucleotide(s) is/are selected from one or more RSV intergenic
3 sequences.

1 191. The isolated polynucleotide molecule of claim 124, wherein said
2 displacement polynucleotide(s) is/are selected from nucleotides within the RSV 5' trailer
3 region.

1 192. The isolated polynucleotide molecule of claim 191, wherein a
2 portion of the 5' trailer region that immediately follows the L gene is reduced in size by
3 75 nucleotides, 100 nucleotides, 125 nucleotides, or more, leaving intact the 5' genomic
4 terminus.

1 193. The isolated polynucleotide molecule of claim 124, wherein said
2 displacement polynucleotide(s) is/are selected from nucleotides within the RSV 3' leader
3 region.

1 194. The isolated polynucleotide molecule of claim 193, wherein a
2 portion of the 3' trailer region that excludes a core portion of the viral promoter located
3 within the first 11 nucleotides of the 3' leader is deleted.

1 195. The isolated polynucleotide molecule of claim 124, wherein a
2 partial or complete deletion from one or any combination of the RSV NS1, NS2, SH, F
3 and/or M2 genes yields an adjustable reduction in genome length of between 1-806
4 nucleotides.

1 196. The isolated polynucleotide molecule of claim 124, wherein a
2 partial or complete deletion from one or any combination of RSV intergenic regions
3 yields an adjustable reduction in genome length of between 1-198 nucleotides.

1 197. The isolated polynucleotide molecule of claim 124, wherein a
2 partial or complete deletion from one or any combination of RSV intergenic regions
3 yields an adjustable reduction in genome length of between 1-198 nucleotides.

1 198. A method for producing an infectious attenuated recombinant RSV
2 particle from one or more isolated polynucleotide molecules encoding said RSV,
3 comprising:

4 expressing in a cell or cell-free lysate an expression vector comprising an
5 isolated polynucleotide comprising a recombinant RSV genome or antigenome having
6 one or more shifted RSV gene(s) or genome segment(s) within said recombinant genome
7 or antigenome that is/are positionally shifted to a more promoter-proximal or promoter-
8 distal position relative to a position of said RSV gene(s) or genome segment(s) within a
9 wild type RSV genome or antigenome, and RSV N, P, L and RNA polymerase elongation
10 factor proteins.

1 199. The method of claim 198, wherein the recombinant RSV genome
2 or antigenome and the N, P, L and RNA polymerase elongation factor proteins are
3 expressed by two or more different expression vectors.

1 200. An isolated infectious chimeric respiratory syncytial virus (RSV)
2 comprising a major nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P), a large
3 polymerase protein (L), a RNA polymerase elongation factor, and a partial or complete
4 bovine RSV background genome or antigenome combined with a plurality of
5 heterologous gene(s) and/or genome segment(s) of a human RSV selected from
6 heterologous gene(s) and/or genome segment(s) of RSV NS1, NS2, M, SH, G, and/or F,
7 to form a human-bovine chimeric RSV genome or antigenome.

1 201. The isolated infectious RSV of claim 200, wherein both human
2 NS1 and NS2 genes are substituted for their bovine counterpart NS1 and NS2 genes.

1 202. The isolated infectious RSV of claim 201, which is rBRV/A2-
2 NS1+2.

1 203. The isolated infectious RSV of claim 200, wherein human NS1,
2 NS2, G, and F are substituted for their bovine counterpart NS1, NS2, G and F genes.

